OBJECTIVES OF COAL BIOPROCESSING AND APPROACHES

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INTRODUCTION

There has been a great deal of excitement about various practical applications of biotechnology, including the production of chemicals, fuels, foods, and drugs; waste treatment; clinical and chemical analyses; toxicological assays; and uses in medicine. In fact, in the last four years there have been four issues of *Science* devoted to this "revolution" in biology. The two most recent were entitled "Biological Frontiers" (1) and "Biotechnology" (2).

Microbe-catalyzed processes constitute industrial microbiology, which today is a diversified multi-billion dollar industry (3,4). The applications of living microbial, plant and animal cells for the production of useful compounds has been extensive. The microbial conversions of lignocellulosic material, the biological precursor to coal, have also been studied in detail.

Studies in coal bioprocessing, however, is just getting underway and still in its infancy. Conceptually coal bioprocessing can be categorized into two areas: 1) coal cleaning--removal of undesirable components such as sulfur, nitrogen, trace metals; and 2) coal conversion--microbial liquefaction, microbial gasification, microbial pretreatment, methane production. The ability of any microbe or microbial consortia to break down a complex structure depends on the types of chemical bonds and the three dimensional environment around the bonds. Therefore, intertwined with coal bioprocessing is an understanding of the coal macromolecular structure and biochemical mechanisms by which microbes/enzymes break bonds. These relationships are depicted in Figure 1.

The objectives of microbial coal cleaning are clear. Removal of sulfur, nitrogen and trace metals by mild microbial processes for the production of clean coal is the overall goal. However, in the area of microbial coal conversion it seems that the objectives are not very clear. For example, in the case of microbial lignin degradation the objective is very clear; that is the complete degradation of the lignin. Obviously, one does not want to completely degrade the coal, but convert the coal into a more usable form by the application of microbial transformations.

Thus, the overall objective in any coal conversion scheme, whether microbial or chemical, is to produce a better fuel form, be it liquid fuel (transportation fuels), solid fuel, or even a new fuel form for direct utilization. This essentially entails depolymerizing of the coal macromolecule, removal of oxygen, increase in H/C ratio. This concept is depicted in Figure 2. It must be borne in mind that unlike in the lignin degradation, the coal can not just be subjected to a non-specific degradation, but must undergo microbial transformations resulting in a depolymerized, deoxygenated, denitrated product which would result in a better fuel.

The area of microbial conversion of coal is receiving a lot of attention these days. Cohen and Gabriel (5) reported that fungi could grow directly on and metabolize naturally occurring coal. Scott (6), Wilson (7), Ward (8), and Faison (9) have also reported degradation or solubilization of lignites by various fungal strains including the solubilization of a Wyodak subbituminous coal (6). However, little is known about the products of the degradation or solubilization. As

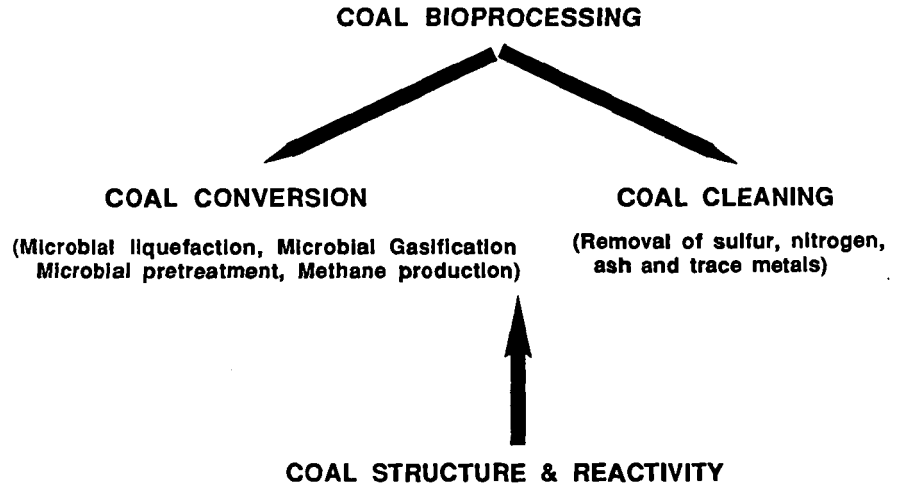

Figure 1. Coal bioprocessing areas

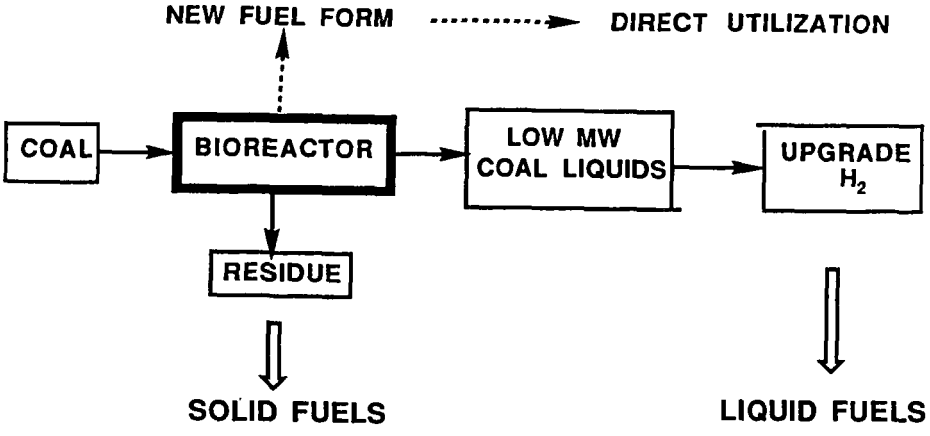

Figure 2. Coal bioprocessing schematics

indicated earlier, the overall objective in any coal conversion scheme whether microbial or chemical is to produce a better fuel form.

Towards achieving this goal, there are two basic approaches to microbial processing of coal. The first approach involves the oxidative solubilization or depolymerization of the coal macromolecule. It involves using enzyme systems or microorganisms to hydroxylate aromatic rings followed by ring fission to a carboxylic acid. Since all of this involves introduction of oxygen into the coal, the question of whether coal is being converted to a less desirable fuel form (converting coal into biomass?) becomes an important issue. On the other hand, if the oxygenated material is more receptive to deoxygenation and/or upgrading than the starting coal, or has other uses, then this type of microbial conversion makes sense.

A second approach to microbial processing of coal, which, according to me promises to be much more rewarding and exciting. The approach envisions using facultative *anaerobic bacteria* to reductively depolymerize/solubilize coal, i.e., reduction of aromatic rings and reductive cleavages to product a hydrogenated product. In other words, hydrogenation of coal via an anaerobic microbial process resulting in a more desirable fuel form rather than oxidation via an aerobic microbial process which results in a less desirable fuel form. Indeed, coal scientists throughout the ages have been trying to do exactly this; i.e., inexpensive approach to product a depolymerized, hydrogen-rich coal fuel.

MICROBIAL COAL TRANSFORMATION IN AEROBIC SYSTEMS
(Oxidative Solubilization/Depolymerization)

All of the microbial conversions (degradation or solubilization) reported so far in literature involves aerobic systems. The mechanism operating under these conditions is oxidative, depolymerization that is hydroxylation of the aromatic ring followed by ring scission. Indeed, it has been shown that biodegradation/oxidation of aromatics are initiated by a series of enzymes known collectively as the oxygenases. The oxygenases can be further sub-divided into dioxygenases and monoxygenases. Molecular oxygen is essential for them to function since it is incorporated into the end product. The reaction pathway is shown in Figure 3 (10) with ring cleavage occurring at the bond between the hydroxyls (orthocleavage) or the bond adjacent to the hydroxyl. Two- (11,12) and three-ring aromatics (13-15) can also be degraded although free ortho ring positions must be available (Figure 4). Low-ranked coals have a number of hydroxy and dihydroxy aromatic structures, as well as carboxy groups. *Pseudomonas* species are capable of carrying out oxidation ring cleavage reactions (16-20) (Figure 5), or aromatic rings containing these functional groups. Therefore, the *Pseudomonas* species should be capable of oxidizing low-rank coals. It would be interesting to compare the products obtained from the microbial oxidative cleavage reactions with that obtained from the ruthenium tetraoxide catalyzed oxidation of the coal. This is because the mechanism of the ruthenium tetraoxide oxidations involves hydroxylation of the aromatic rings followed by ring cleavage which is similar to the mechanism of microbial oxidative ring cleavages.

Again, the question that pops up is whether such microbial processing approaches for depolymerization/solubilization of the coal makes sense. The reason being that we have introduced a number of oxygen functional groups during the process, which could result in less desirable fuel qualities.

Lignin Biodegradation

Lignin is considered as the precursor to coal and some young lignites may contain 35%-70% of lignin-like compounds. It is, therefore, not surprising that lignin degrading microorganisms and enzymes are being employed for microbial coal conversion. For example *Phanerochaete chrysosporium* and *Polyporous versicolor* have been used in microbial coal conversions.

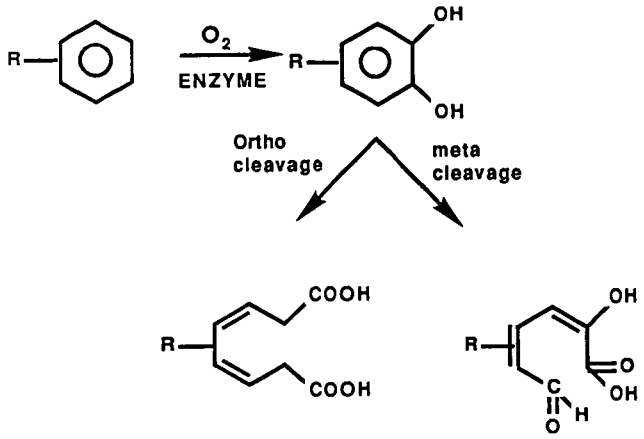

Figure 3. Mechanism of aromatic degradation by oxygenases

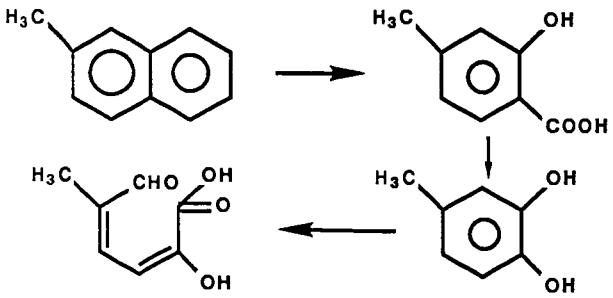

Figure 4. Degradation of two ring compounds

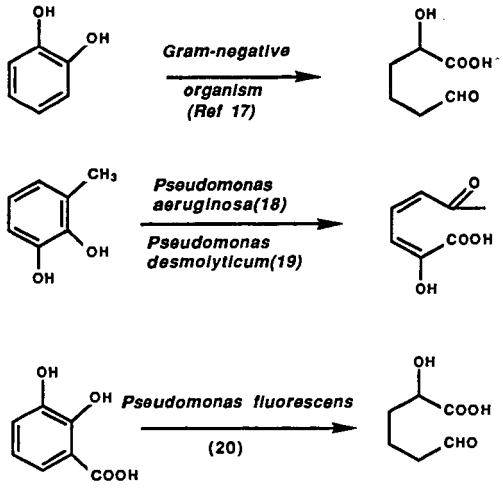

Figure 5. Oxdative ring opening reactions by *Pseudomonas*

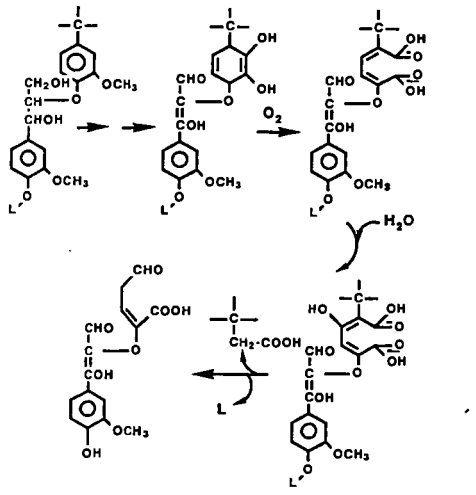

Figure 6. Mechanism of lignin degradation

These are "white rot" fungi and their mechanism of action for lignin degradation involving the enzymes ligninase and laccase have been extensively studied by Kirk and others (21-23). They report that the degradation proceeded by non-specific hydroxylation and ring opening just like the oxygenases functions and, not as previously believed, by beta-arylether cleavage initiated by a specific etherase enzyme (Figure 6).

ENZYMES VS WHOLE CELLS

The question also arises as to whether coal bioprocessing would be better achieved with growing cells or isolated enzymes. Multistep transformations such as the synthesis of interferon or the production of ethanol from cellulose involves a number of different enzymes acting sequentially and regeneration of co-factors is required. Therefore, it is clearly advantageous to use whole cells. For one step or two step transformations however, enzymes are probably superior because their use is free of drawbacks such as competing side reactions, sterility problems, and the cell lysis often associated with fermentations. Enzyme systems like the ligninases, oxygenase could promote selective transformations. There also exists the capability of overproducing the coal processing enzymes using recombinant DNA or genetic engineering techniques. In fact, the ligninase enzyme has been cloned in *Escherichia coli*. It therefore seems to me that the use of isolated enzymes for coal bioprocessing is advantageous and should be pursued.

In our laboratory, we are evaluating enzymes present in various microorganisms to carry out decarboxylation, hydrogenation and non-oxidative depolymerization of the coal. Preliminary results have shown that microorganisms such as *Bacillus megaterium* containing decarboxylase(s) can remove CO_2 from *model compound* vanallic acid and coal. The resulting coal has a higher H/C ratio. Low rank coals have a substantial number of oxygen tied up as carboxyls.

ANAEROBIC BIOCONVERSION OF COAL (Reductive Depolymerization/Solubilization)

A more exciting and potentially rewarding field is the area of coal bioprocessing under anaerobic conditions. To the best of our knowledge, almost no work has been done in this area. This approach envisions the use of facultative anaerobic bacteria to reduce the aromatic ring systems in the coal and produce a depolymerized hydrogenated product. In other words, instead of adding oxygen via an aerobic process and producing a less desirable fuel, we can add hydrogen to the coal using an anaerobic process and produce a richer fuel.

Anaerobic Aromatic Ring Metabolism

Under anaerobic conditions, aromatics are degraded by either hydration or hydrogenation followed by non-oxidative ring fission. In all cases investigated the microorganisms initially reduce the ring structure (24). Tarvin and Buswell (25) reported the complete utilization of benzoate, phenylacetate, phenyl propionate and cinnamate by anaerobes. Healy and Young (26) found that 11 simple lignin derivatives were biodegraded to methane and carbon dioxide under strict anaerobic conditions. Anaerobic degradation of two and three ring lignin fragments has been proposed to occur via an overlap of the ferulate and benzoate degradative pathways (27).

Cleavage of the benzene nucleus anaerobically occurs by at least three different reaction schemes as shown in Figure 7.

A. Photometabolism; ex *Rhodopseudomonas palustris*..

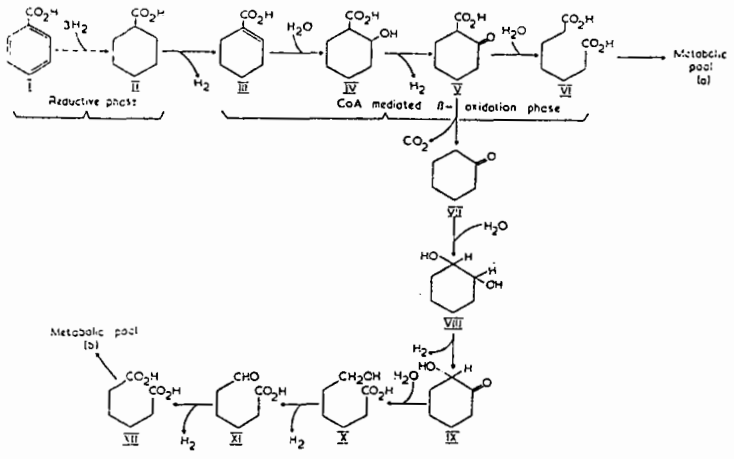

Figure 7. Anaerobic aromatic - ring metabolism

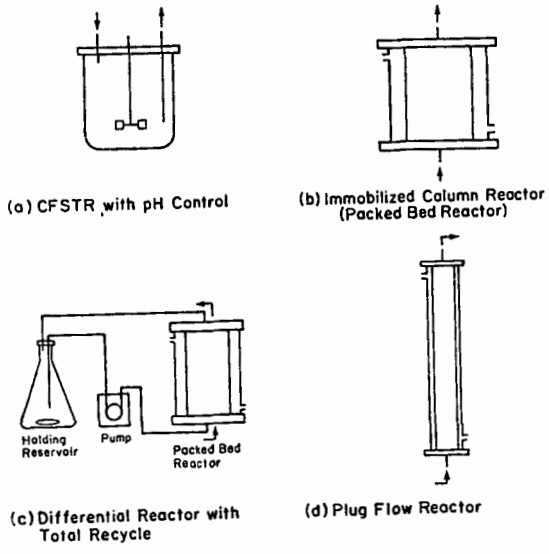

Figure 8. Bioreactor configurations.

B. Nitrate respiration; ex *Moraxella sp.*

C Methanogenic fermentation; ex occurs via a consortium of gram negative organisms and various methane bacteria.

Several species of the purple non-sulfur bacteria, the *Rhodospirillaceae*, are able to use aromatic compounds as sole carbon sources by photometabolism. When cell suspensions of *R. palustris were* incubated with (^{14}C) benzoate the seven carbon atoms of benzoate remained together until after the ring cleavage stage. These results suggested a series of reactions involving the reduction of benzoate (or a derivative) to a cyclohexanecarboxylate moiety followed by a coenzyme-A mediated beta-oxidation sequence. Because the reduced acid is acyclic, breakage of the bond in the 1,2 position occurs to yield pimelate (or its equivalent). This new reductive pathway is illustrated in Figure 7b. Evans (28) has recently demonstrated the occurrence of these reactions using subcellular fractions from *R. palustris* grown photosynthetically on benzoate with the following results: (a) a washed chromatophore or benzyl-Co A under illuminated, anaerobic conditions; (b) a cell-free extract in the presence of CoA, ATP, NAD^+ and Mg^{2+} ions converted cyclohex-1-enecarboxylate to pimelate in anaerobic or aerobic conditions in the light or dark.

These results provide strong evidence for the presence of a light-dependent membrane bound proton-translocating redox system in these chromatophores; the low potential reductant may be a ferrodoxin, which also plays a part in photosynthetic electron transport. In addition, they confirm the presence of the appropriate beta-oxidation suite of enzymes in these cells responsible for the subsequent series of reactions resulting in ring-cleavage.

This is an exciting approach to the bioconversion of coal. One can take advantage of the capability of phototrophic bacteria for reductive bioconversion of aromatics to carry out coal bioconversions.

MICROBIAL COAL CLEANING

Unlike microbial conversions, the microbial cleaning objectives are much better defined. Removal of sulfur primarily trace metals, chlorine and nitrogen by microorganisms is the focus of attention. Microbial desulfurization, especially, pyrite removal has received a lot of attention, and a number of papers by different research groups have been published on the subject (29,30). *Thiobacillus* is used for pyritic sulfur removal and a host of literature is available on the subject. The question arises whether it is cost-effective to use *Thiobacillus* or other microorganisms for pyritic sulfur removal because it takes weeks and even months for the reaction to go to completion. A more viable option in which Atlantic Research Corp. and Illinois Geological Survey, are working in the use of microorganism to surface modify the pyrite is coal and make it more hydrophilic. This type of conditioning takes only three to four hours and a conventional flotation process can now eliminate almost all of the pyritic sulfur from this microbially pretreated coal. Another approach would be to let loose the microorganism in a coal storage pile and then come back later to have a sulfur-free coal available. The idea is not as far fetched as one thinks. Indeed, in Cuba, such an approach is used for the removal of lignin from cane sugar--called the Cubanine process. Lignin-degrading enzymes from the white rot fungus are put together with the biomass cane sugar material in a large chamber and after a month has elapsed the biomass is taken up, ground and made ready for use. The Cubans have found that this method is cost-effective in removing lignin and saves them a lot of energy which they would, otherwise, have to expend to remove the lignin.

BIOPROCESS ENGINEERING COMPONENT

Finally, one has to recognize that in order for these microbial conversions to reach a commercial scale, bioprocess considerations are warranted. Bioreactor design, problems about nutrient recycle or other process considerations entails the fact that bioreactor engineering will have to become an integral component of any coal processing scheme. Figure 18 illustrates some of the possible bioreactor configurations that can be used.

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